

Effects of Subchronic Exposures to Concentrated Ambient Particles (CAPs) in Mice: IV. Characterization of Acute and Chronic Effects of Ambient Air Fine Particulate Matter Exposures on Heart-Rate Variability

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Long-term exposure to fine particulate air pollution (PM_{2.5}) has been associated with increased risk of death from cardiopulmonary diseases. Cardiac function parameters have also been affected by ambient particulate matter (PM) exposure, including heart-rate variability (HRV), a measure of autonomic function that has been recognized as a well-defined, quantitative indicator of autonomic dysfunction. However, the role of HRV in ambient PM-induced cardiovascular effects is not fully understood. In an accompanying article, we report significant decreasing patterns of heart rate (HR), body temperature, and physical activity for mice lacking apolipoprotein (ApoE^{-/-}) over 5 mo of exposure to concentrated ambient PM (CAPs), with smaller and nonsignificant changes for C57 mice. In this article, we report the effects of subchronic CAPs exposure on HRV parameters that are sensitive to cardiac sympathetic and parasympathetic nerve activity. The standard deviation of normal to normal beat intervals (SDNN) and the square root of the mean squared differences of successive RR intervals (RMSSD) in the late afternoon and overnight for the ApoE^{-/-} mice showed a gradual increase for the first 6 wk, a decline for about 12 more wk, and a slight turn upward at the end of the study period. For C57 mice, there were no chronic effect changes of SDNN or RMSSD in the late afternoon, and a slight increase after 6 wk for the overnight period. The response patterns of ApoE^{-/-} mice indicated a perturbation of the homeostatic function in the cardiovascular system (initial enhancement and later depression of the HRV parameters). Our results complement the findings in human panel and controlled CAPs exposure studies in demonstrating that increased levels of particle pollution are able to perturb cardiac autonomic function, which may lead to adverse cardiovascular outcomes.

Several large prospective cohort mortality studies (Dockery et al., 1993; Pope et al., 1995, 2002; Laden et al., 2001) have shown that long-term exposure to fine particulate air pollution (PM_{2.5}) is associated with increased risk of death from cardiopulmonary diseases, and a very recent publication on the largest cohort studied to date demonstrates that the risk is especially high for mortality via mechanisms involving inflammation, ac-

celerated atherosclerosis, and altered cardiac autonomic function (Pope et al., 2004b). Other recent studies focusing on acute effects of ambient particulate matter (PM) on the cardiovascular system have shown that changes in blood viscosity (Peters et al., 1997), changes in heart rate (Pope et al., 1999; Gold et al., 2000), and alterations in heart-rate variability (HRV) (Liao, 1999; Pope & Dockery, 1999; Gold et al., 2000) are all associated with increases in PM exposures. In patients with implanted cardiac defibrillators, associations were reported between PM and increased defibrillator discharges (Peters et al., 2000). In another study, the risk of onset of myocardial infarction (MI) was increased in association with PM levels in the 2 h preceding the MI (Peters et al., 2001).

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Among the cardiac parameters that were affected by ambient PM exposure, HRV, a measure of autonomic function of the cardiovascular system, has been recognized as a well-defined, quantitative indicator of autonomic dysfunction in cardiovascular mortality (Task Force, 1996). There is general agreement that reduced HRV is an unfavorable prognostic marker for cardiovascular disease (Task Force, 1996). Recent studies had found associations between ambient particle levels and reduced HRV (Liao et al., 1999; Pope et al., 1999), suggesting that particle air pollution may increase cardiovascular risk by perturbing autonomic function. However, the precise roles of HRV in ambient PM induced cardiovascular effects are not fully understood. For example, although alterations in heart rate (HR) and other cardiac parameters in animals exposed acutely to ambient PM (Nadziejko et al., 2002a, 2002b; Cheng et al., 2003; Godleski et al., 2000; Vincent et al., 2001), residual oil flyash (ROFA) (Wellenius et al., 2002; Godleski et al., 2000), diesel exhaust (Campen et al., 2003), and other laboratory-generated aerosols (Nadziejko et al., 2004b; Campen et al., 2001, 2002) have been reported, few of these studies focused on the long-term effects of particles on cardiovascular system including HRV. The lack of prolonged CAPs exposure studies hampers the significance of the findings of the large cohort studies (Pope et al., 1995, 2002, 2004; Dockery et al., 1993; Laden et al., 2001), which imply that the mortality impact of long-term elevated PM exposure is several times greater than that indicated by the daily time-series mortality studies. It is therefore evident that one of the greatest needs for additional research was in the area of chronic animal inhalation studies with PM_{2.5}.

Within the overall subchronic study conducted at New York University (NYU) in 2003, and presented elsewhere in this issue of *Inhalation Toxicology* (Hwang et al., 2005), there were significant decreasing patterns of HR, body temperature, and physical activity for the mice lacking apolipoprotein E (ApoE^{-/-}) over 5 mo of exposure to concentrated ambient PM (CAPs), with smaller and nonsignificant changes for the C57 mice. There was also a significant relationship between CAPs exposure concentration and short-term change of heart rate in ApoE^{-/-} mice during the daily CAPs exposure. Response variables were also defined for examining fluctuations of 5-min heart rates within long (i.e., 3–6 h) and short time periods (i.e., ~15 min). According to the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Task Force, 1996), the fluctuation of HR with the cycle length of 3–6 h could be influenced by circadian rhythm, and those with cycle length of 15 min could be affected by temperature and humoral systems. In this article, we report the effects of subchronic CAPs exposure on HRV parameters that are sensitive to cardiac sympathetic and parasympathetic nerve activity.

EXPERIMENTAL DESIGN

This study is part of a subchronic CAPs exposure study investigating the effects of CAPs on cardiovascular system. The rationale and experimental design of this study are detailed else-

where in this special issue (Lippmann et al., 2005). In this study, the normal mice (C57) and knockout transgenic mice lacking apolipoprotein E (ApoE^{-/-}) were randomly assigned into control and exposure groups. C57 mice were obtained from the Jackson Laboratory (Bar Harbor, ME), and ApoE^{-/-} mice were obtained from Taconic Europe (Denmark). The mice with implanted transmitters were housed singly in our ALAAC-accredited animal housing facility at Tuxedo, NY, and the electrocardiograph (EKG) parameters were monitored continuously. Starting at 7 mo before the start of the CAPs exposures, ApoE^{-/-} mice were fed a high-fat diet (Adjust Calories Diet, TD88137, Harlan, Indianapolis, IN) for 4 mo. Severe skin irritation developed in some of these mice, and all ApoE^{-/-} mice were switched to a normal diet 3 mo prior to the CAPs exposures. The C57 mice were on a regular diet throughout, and had access to food and water ad libitum.

Animals were exposed to northeastern regional background CAPs using a modified VACES system. The detailed design and performance of the entire system as well as exposure atmosphere characterization are described elsewhere in this special issue of *Inhalation Toxicology* (Maciejczyk et al., 2005). Briefly, the exposures were conducted in our Sterling Forest laboratory in Tuxedo, NY, 40 miles northwest of Manhattan. Animals were exposed to filtered air or CAPs at 10× ambient concentrations for 6 h/day, 5 days/wk. The exposures started on April 11, 2003, and ended on September 5, 2003, but EKG monitoring continued for 5 nonexposure days to September 10, 2003. The animals with implanted EKG telemeters were exposed to either filtered air (control group) or CAPs (exposure group) around 9:00–15:00 on weekdays and stayed in an animal room with filtered air for the rest of the time, except for short time periods required to move them in and out of the exposure chambers. Ten-second averages of heart rate and body temperature were obtained every 5 min. Physical activity counts for every 5-min interval were expressed as counts per minute, as described by Hwang et al. (2004). There were 9 ApoE^{-/-} mice in the control group and 10 in the exposure group. Although the experiment started with six C57 mice in each group, due to battery failure and some mechanical problems, complete data for three of the mice in this exposure group were not available for analysis. The light in the animal room was turned on at 6 a.m. and off at 6 p.m. automatically to control the living environment consistent over the 5 mo of experiment. Due to some undetected mechanical problems, the light was on 24 h/day during late June and early July, and this event may have had impacts on heart rate, physical activity, and body-temperature measurements for that time interval and possibly beyond.

DATA ANALYSIS

The analysis of acute and chronic effects on heart rate and body temperature of the study was described in detail elsewhere in this issue (Hwang et al., 2005). The EKG data from 9 C57 and 19 ApoE^{-/-} mice were retrieved for heart-rate variability analysis. The average of CAPs mass concentrations during each

day's 6-h exposure was calculated for use in examining any acute concentration-response effects.

The times in milliseconds of occurrence of two consecutive R waves in EKG channel (RR) were calculated on a beat-to-beat basis using the computer package of Dataquest A.R.T. Analysis. Due to limitation in data storage capacity, the RR intervals were recorded consecutively for 5 s in every 15-min interval for all mice during April 10–27, 2003, and for ApoE^{-/-} mice in the control group during April 22–July 20, 2003. The rest of recordings were taken consecutively about 10 s in every 5-min interval for the mice. There are about 34–64 and 100 RR intervals recorded in 15-min and 5-min intervals, respectively. For the analysis, we decided to work on fluctuations of RR intervals on an every 15 min basis. To match the data in the 15-min recordings, we used only the first 60 RR intervals in the last of 3 consecutive 5-min intervals. The two heart-rate variability (HRV) indices that we used were SDNN, the standard deviations of the RR intervals, and RMSSD, the square root of the mean squared differences of successive RR intervals in 5 s; these were calculated for each 15-min interval and each mouse. Preliminary analyses of time series of these 15-min HRV measurements of ApoE^{-/-} mice showed that measurements of HRV at night tended to be higher in the CAPs exposure group than in the control group in June. The pattern was in the opposite direction after late July. To clarify the circadian pattern changes of the HRV measures for the two groups, and to identify a time period for examining chronic changes of HRV, we applied the nonparametric method to the variables during the 60-h period beginning at 18:00 each Friday and continuing to 06:00 on Monday (Nadziejko et al., 2004a). Based on the data for ApoE^{-/-} mice in the first 3 weekends in June, the nonparametric method identified the time period 00:00–05:00 during which the 2 groups had the largest HRV differences within each day. To match the analyses of effects on body temperature and heart-rate changes in this subchronic effects study, we decided to use the same time period (1:30–4:30 a.m.) for calculating mean log SDNN and log RMSSD to represent daily HRV responses for this time period for each mouse. In the analysis of effects on heart rate and body temperature, we calculated daily responses for the time period of 11:00–13:00 during exposure for examining acute effects. However, since many RR intervals recorded during the exposure time period were of questionable quality, we picked the 16:00–18:00 time interval after exposure instead as an alternate for calculating daily HRV response.

STATISTICAL MODEL

A two-stage Bayesian hierarchical model was developed to estimate chronic changes of heart rate, activity counts, and body-temperature responses and is reported in detail elsewhere (Hwang et al., 2005). In the first stage, the crude effects of CAPs on log SDNN and log RMSSD on the ApoE^{-/-} and C57 mice were estimated for each day from a time-varying regression model. For each combination of time period, HRV measure, and mouse, we assumed that the daily series of crude effects z_t for

the case consist of a smooth function of chronic effects and acute effects that may be affected by current daily exposed CAPs levels and an error term. Specifically, in stage two of the Bayesian hierarchical model, we assume that the estimated crude effect z_t is normally distributed with unknown mean θ_t and known standard deviation $\hat{\sigma}_t$, which is the standard error of z_t obtained in stage one. The daily series of effect parameters $\theta_1, \theta_2, \dots, \theta_n$ are further modeled by $\theta_t = \sum_{k=0}^p \alpha_k \cdot \max(0, t - \omega)^k + (\beta_0 + \beta_1 \cdot X_t) \cdot I_t + \phi \cdot L_t + e_t$, where ω is the onset of chronic effect, X_t is the daily log CAPs concentration, I_t is an indicator function for exposure day, L_t is another indicator function for the days of light system failure, and the error term e_t is an autoregressive process of some unknown order. Priors of these parameters are all noninformative. The final model including the orders of the polynomial function and autoregressive process is determined by the Bayesian deviance information criterion (DIC). Posterior samples of the parameters are generated using the free package WinBUGS (WinBUGS Project, 2004, <http://www.mrc-bsu.cam.ac.uk/bugs/>). Posterior mean and 95% credible interval (C.I.) of the polynomial function are calculated for estimation of chronic effect change on HRV. The parameter β_1 describes the relationship between daily exposure mass concentration and acute effect change. If β_1 is not significantly from 0, β_0 itself can be interpreted as the effect of exposure day against nonexposure day. The parameter ϕ is used for adjusting different impacts on mice in exposure and control groups of the room light system failure that went undetected for several weeks during the exposure period.

RESULTS

The crude effects in log SDNN and log RMSSD changes for the two time periods of 16:00–18:00 and 1:30–4:30 estimated from the stage one models for ApoE^{-/-} mice are illustrated in Figures 1 and 2. The closed circles are for crude effect estimates on exposure days, while open circles are for nonexposure (mainly weekend and holiday) days. For each case, we computed the deviance information criterion (DIC) for different orders of the polynomial function, and chose the order with smallest DIC as the final model. Figure 1 shows the posterior means and 95% C.I.s of the chronic effect changes of log SDNN at these two time periods for the ApoE^{-/-} mice. The exposure effect on log SDNN changes at 16:00–18:00 showed a gradual increase for the first 6 wk, a downward trend for about 12 more wk, and a slight turn upward to the end of the study. The chronic effect change pattern for the log SDNN at 1:30–4:30 was similar to that of 16:00–18:00. The main difference was that the peak was higher at the time period of 1:30–4:30. For the log RMSSD, the two estimated plots of chronic effect changes at the two time periods shown in Figure 2 were very similar to those of log SDNN. For C57 mice, the log SDNN measure, shown in Figure 3, exhibited no chronic effect changes at the time period of 16:00–18:00. For the time period of 1:30–4:30, there was a slight increase after 6 wk. The log RMSSD effect measures (Figure 4) were similar to those of log SDNN measure for the two time periods.

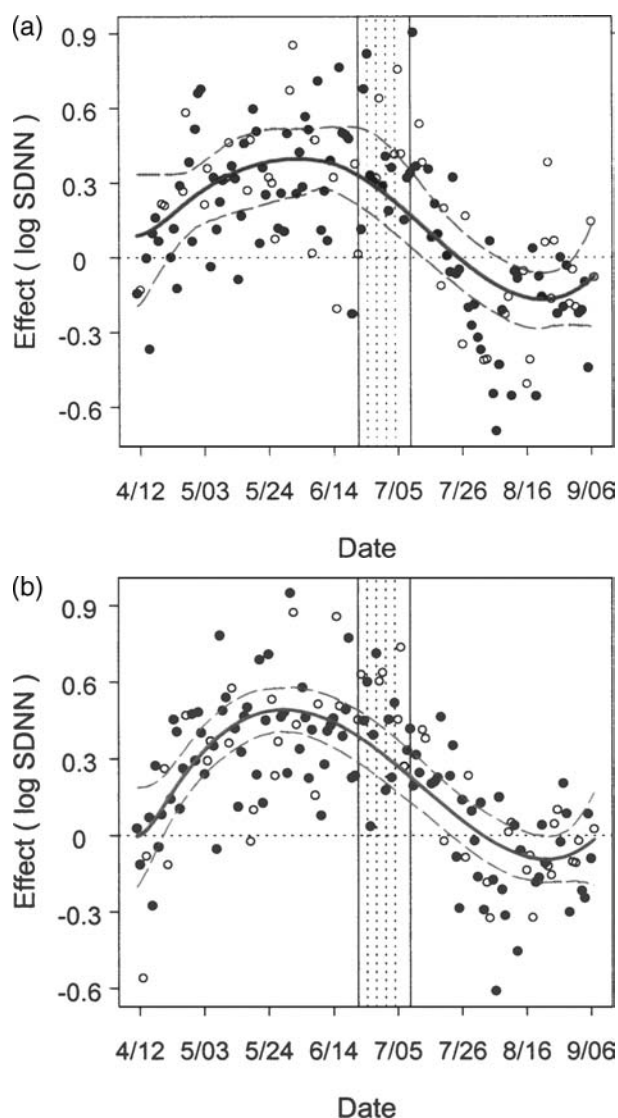


FIG. 1. The posterior mean (solid) and 95% C.I. (dotted) of chronic effect changes of log SDNN for ApoE^{-/-} mice during (a) 16:00–18:00 and (b) 1:30–4:30 obtained from the Bayesian model in stage two. The circles in the plots are daily crude effects estimated in stage one (full for exposure day and empty for nonexposure day). The light failure dates determined in the model are marked with vertical bars.

As shown in Table 1, the posterior means and 95% C.I.s. of the acute parameters β_0 and β_1 for all the eight cases indicated that no significant acute effects were found to be related to the CAPs exposed concentrations at these two time periods. Since the lesser quality of RR measurements during the exposure period did not allow us to establish the possible acute effects at the time of exposure, we explored the possible longer lag effects by examining the relationships between CAPs concentrations on Fridays versus estimated acute effects at 0130–0430 and 1600–1800 on Saturday and Sunday. As shown in Figure 5, no clear

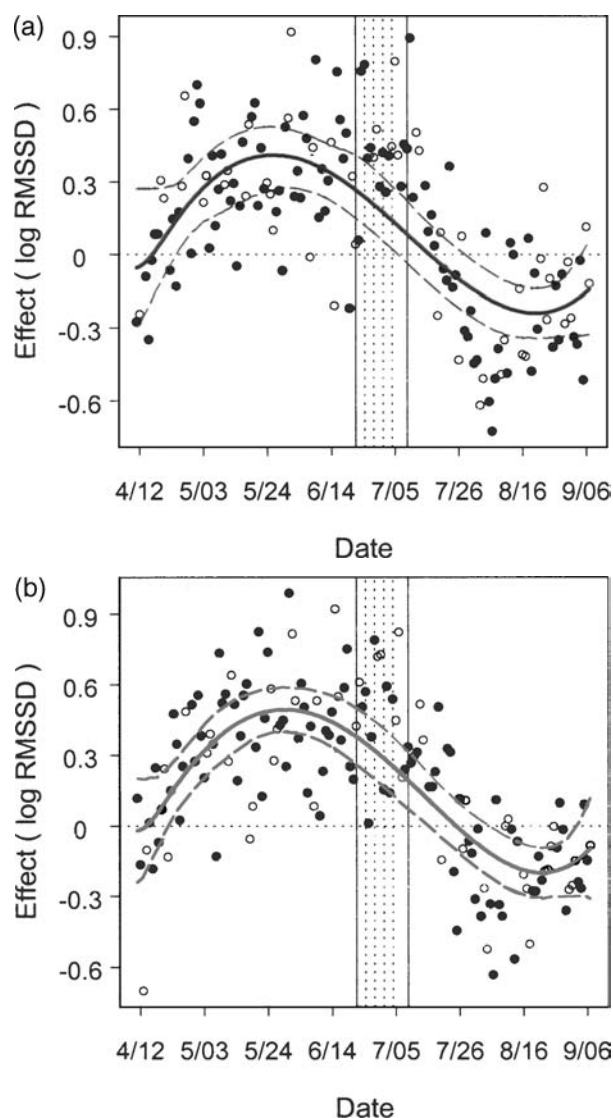


FIG. 2. The posterior mean (solid) and 95% C.I. (dotted) of chronic effect changes of log RMSSD for ApoE^{-/-} mice during (a) 16:00–18:00 and (b) 1:30–4:30 obtained from the Bayesian model in stage two.

pattern was seen between CAPs concentrations and estimated acute effects with the 48-h time period postexposure. However, as reported in Maciejczyk and Chen (2005), the changes in HRV in the ApoE^{-/-} were correlated to the compositions of CAPs.

The posterior mean (95% C.I.) of the parameter for light failure adjustment were 0.16 (0.02, 0.3) and 0.18 (0.03, 0.33) for ApoE^{-/-} mice measures in log SDNN and log RMSSD at 1:30–4:30. From the original data and the positive estimates we found that the light failure had reduced HRV of mice more in the control group than in the exposure group. We also examined the residuals for the error term in the stage two model and concluded that the error terms were best modeled by independent normal distributions.

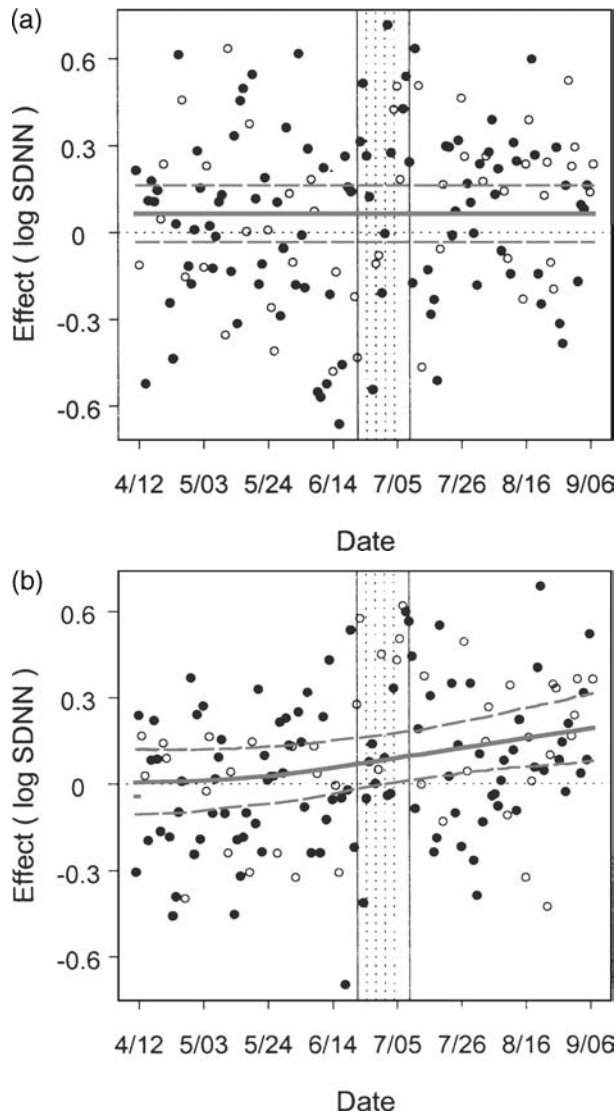


FIG. 3. The posterior mean (solid) and 95% C.I. (dotted) of chronic effect changes of log SDNN for C57 mice during (a) 16:00–18:00 and (b) 1:30–4:30 obtained from the Bayesian model in stage two.

DISCUSSION

In this article, the HRV (both SDNN and RMSSD) of animals that are prone to develop atherosclerosis ($\text{ApoE}^{-/-}$) was affected by subchronic exposure of CAPs at an average exposure concentration of $110 \mu\text{g}/\text{m}^3$ during the 5-mo period ($19.7 \mu\text{g}/\text{m}^3$ overall long-term average). There were no acute changes in these parameters in these animals, nor were any acute or chronic effects seen in normal C57 mice exposed to the same atmospheres. Changes in HRV are indicative of adjustments of the homeostatic balance between sympathetic and parasympathetic components of the autonomic nervous system (Task Force, 1996). The response patterns we observed in $\text{ApoE}^{-/-}$ mice indicated a perturbation of the homeostatic function in the cardiovascular

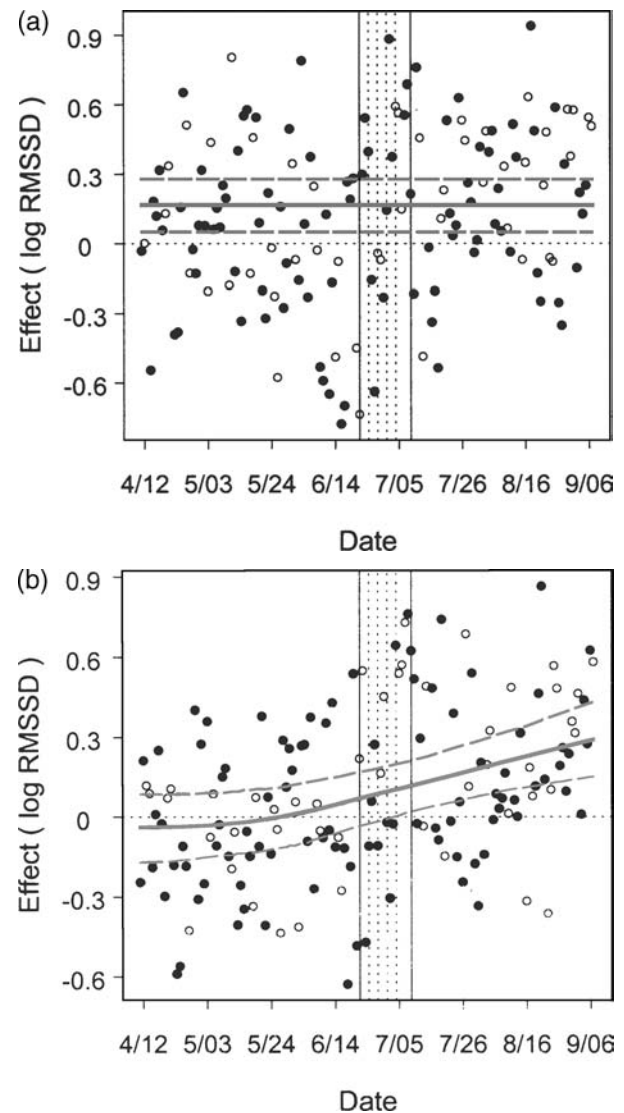


FIG. 4. The posterior mean (solid) and 95% C.I. (dotted) of chronic effect changes of log RMSSD for C57 mice during (a) 16:00–18:00 and (b) 1:30–4:30 obtained from the Bayesian model in stage two.

system with initial stimulation (enhancement) and later depression of the HRV parameters. Although we cannot find any report of shifting HRV patterns in long-term animal exposure studies, moderation of acute hypoxia induced sympathetic activation has been reported in animals maintained at 475 torr (simulated altitude of 4000 m) (Melin et al., 2003). This indicates that analysis of HRV pattern change could be an effective method to assess cardiovascular system control in animals exposed to environmental agents over a long duration.

Bidirectional response to a low-level pollutant challenge in a biological system is not uncommon. For example, the rate of clearance of inert particles from the lung conducting airways in response to sulfuric acid and cigarette smoke has been observed

TABLE 1

Posterior mean and the 2.5th and 97.5th percentiles of intercept β_0 and slope coefficient of log CAPs concentration β_1 for the two HRV parameters of ApoE^{-/-} and C57 mice at the two time periods

Parameter	Measure	ApoE ^{-/-} mice				C57 mice			
		log SDNN		log RMSSD		log SDNN		log RMSSD	
		1600–1800	0130–0430	1600–1800	0130–0430	1600–1800	1:30–4:30	1600–1800	1:30–4:30
Intercept β_0	Mean	0.0524	0.0045	-0.0047	0.085	0.0126	-0.0701	0.1157	-0.0648
	2.5%	-0.2608	-0.2629	-0.3427	-0.1962	-0.3475	-0.3833	-0.3298	-0.4409
	97.5%	0.3486	0.2551	0.3286	0.3832	0.3719	0.2405	0.5574	0.2928
Slope β_1	Mean	-0.0227	-0.0068	-0.0052	-0.0222	-0.0096	0.0039	-0.0366	0.0097
	2.5%	-0.0866	-0.0613	-0.0754	-0.0852	-0.0838	-0.0626	-0.1293	-0.0659
	97.5%	0.0426	0.0488	0.0653	0.0374	0.0671	0.0692	0.0602	0.0860

both in humans (Lippmann & Schlesinger, 1984; Lippmann, 2000) and in experimental animals (Chen & Schlesinger, 1983). In addition, a similar bidirectional pattern was also observed for HR in the same ApoE^{-/-} mice exposed to CAPs (Hwang et al., 2005). A transient rise in HR was seen in first month of CAPs exposure, followed by a substantial decline in the second and third month, and a continued depression to the end of the exposure period. In addition, changes in HR fluctuation (HRF), a measure of

variations in HR analogous to HRV, were markedly progressing at the end of the exposure period (Hwang et al., 2005). Since the autorhythmicity of the cardiovascular system is modulated by many factors (Stauss, 2003), our results in HR, HRF, and HRV suggest that prolonged exposure to CAPs maybe necessary to alter the homeostatic function of the cardiovascular system. The need for prolonged CAPs exposure to induce changes in these cardiac parameters also explained, at least in part, the absence

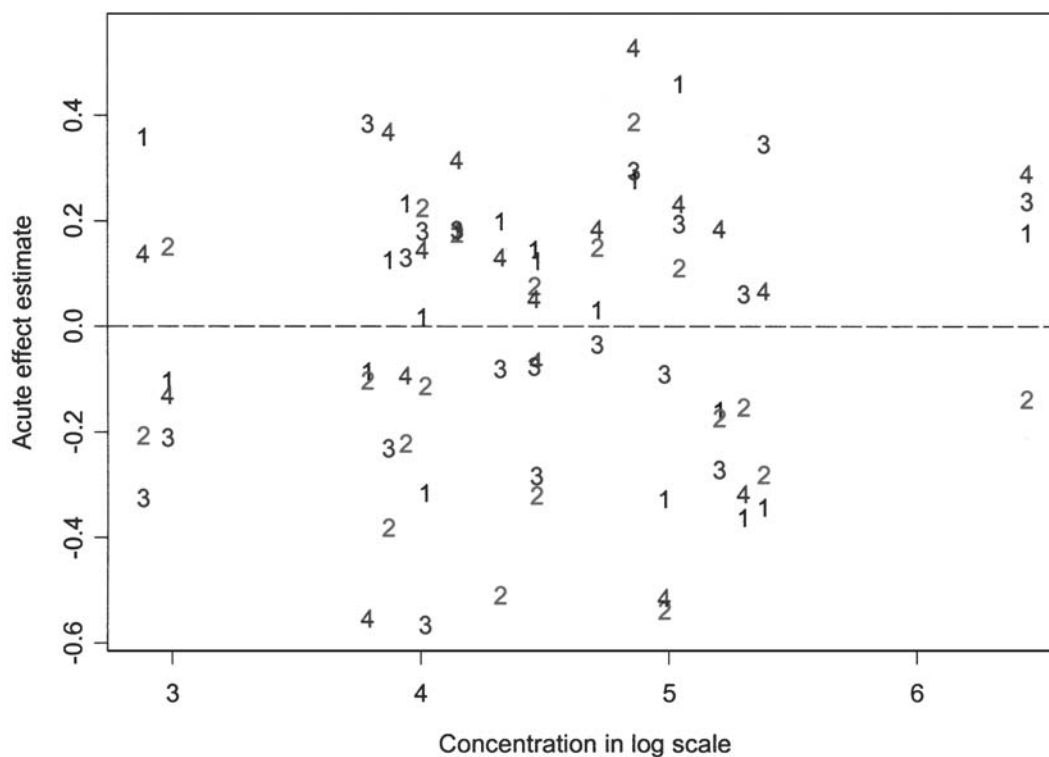


FIG. 5. Scatter plot of CAPs concentration measured on Friday versus estimated crude effects at (1) 0130–0430 Saturday, (2) 1600–1800 Saturday, (3) 0130–0430 Sunday, and (4) 1600–1800 Sunday.

of reported HRV alteration in many of the previous short-term studies involving animal exposures to CAPs and other particles.

Conversely, our results were similar to the observations in human subjects undergoing short-term exposures to ambient particle pollution, perhaps due to the chronic PM_{2.5} exposures of the human populations investigated in these studies. For example, both indoor and outdoor PM_{2.5} were consistently associated with decrease in frequency domain (both low- and high-frequency domains) and time domain (SDNN) variables of HRV in elderly subjects with cardiovascular conditions (e.g., hypertension) in metropolitan Baltimore (Liao et al., 1999). However, a subsequent study with a slightly larger cohort did not report a similar trend (Creason et al., 2001). Similarly, a consistent and statistically robust association of increase in PM_{2.5} and decreases in SDNN and RMSSD was reported for a cohort of 95 subjects with heart disease in Salt Lake City, UT (Pope et al., 2004a), while a smaller cohort of 7 subjects showed inconsistent results (Pope et al., 2004a). Using a protocol involving resting, standing, exercising, and recovering EKG measurements among 21 active elderly subjects in Boston, both HR and RMSSD were associated with elevated PM_{2.5} levels (Gold et al., 2000). Most comparable to the results of our study were the findings that significant decrease in HRV in both time and frequency domains were seen in healthy elderly adults immediately following exposure to 40.5 µg/m³ of Chapel Hill, NC, CAPs, whereas no such effects were observed in young healthy volunteers (Devlin et al., 2003).

In summary, subchronic exposure of north eastern regional background CAPs produced alterations of HRV in animals of compromised health but not in healthy animals. Our results extend the findings in human panel and controlled CAPs exposure studies in demonstrating that increased levels of particle pollution are able to disturb cardiac autonomic function, which may lead to adverse cardiovascular outcomes.

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